

Enzo Therapeutics, Inc.

Amendment to:

Human Gene Transfer Protocol No. 9801-230

BB-IND 7457

Evaluation of the Safety and Effects of *Ex Vivo* Modification and  
Re-infusion of CD34+ Cells by an Antisense Construct Against  
HIV-1 in a Retrovirus Vector

January, 2001

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## SCIENTIFIC ABSTRACT

U937, a CD4+ cell line derived from human promonocytic cells, was made resistant to productive HIV-1 infection by the introduction of a DNA construct that contained 3 independent antisense sequences directed against 2 functional regions, *tar* and *tat/rev*, of HIV-1. Stable transfected cells that expressed all three antisense transcripts were developed. Using these cells we showed that the RNA transcripts accumulated in the nucleus. These transfected cells were subjected to 2 successive challenges with HIV-1 (BAL strain). The surviving cells continued to divide and grow at approximately the same rate and retained their CD4+ phenotype. *In situ* hybridization assays showed that essentially all of the surviving cells produced high levels of antisense RNA. When the cells were challenged with HIV-1 (again BAL strain) no detectable HIV-1 -specific p24 antigen was observed, and PCR-amplified HIV-1 gag nucleic acid sequences were not detected. These data indicated that the presence of intracellular anti-HIV-1 antisense RNA was profoundly inhibiting HIV-1 replication. As a further demonstration that the antisense RNA directed against HIV-1 was present and functioning in these transfected immune cells, *tat*-activated expression of the gene coding for chloramphenicol transacetylase was shown to be specifically inhibited in cells expressing either of the *tat*-antisense sequences or the *tar*-antisense sequence singly. The CD4 antigen was shown to remain on the surface of these highly resistant transfected cells by flow cytometry. Also, the cells containing these three genetic antisense genes did not support the replication of three independently isolated HIV-1 strains (Liu *et al.*, J Virol 71: 4079-4085.1997).

The antisense sequences have been embedded into separate cloned human U1 RNA genes. These three U1/HIV-1 antisense genes were then combined into a triple U1/HIV-1 antisense cassette and incorporated into a Moloney Murine Leukemia Virus (MMLV)-based transducing vector. This murine retrovirus-based vector has the unusual property that it transduces CD34-enriched human cell populations in 18 hours without added cytokines and in the absence of any stromal cell feeder layer as well as it does in the presence of stromal cells and in the presence of IL3, IL6 and SCF and over the course of several days of culturing.

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These data suggested that the presence of our anti-HIV-I genetic antisense RNA in CD4+ cells would be sufficient to manage HIV-1 levels in infected subjects. Accordingly we initiated a clinical trial (Human Gene Transfer Protocol No. 9801-230 of which this protocol is an amendment) in which we transduced PBSC from HIV-1-infected subjects using our transducing vector HGTV43 and infused them autologously. The end points of the phase 1 trial were to test for the safety of this procedure and to test for engraftment and differentiation of the transduced cells and secondarily to test whether multiple infusions of transduced cells led to a higher extent of engraftment when compared with a single infusion.

In this trial, in all subjects infused with transduced autologous PBSC, the numbers of viable PBSC induced and infused were within generally accepted ranges for HIV-1 and the reported data on transduction with retrovirus-based transducing vectors.

No serious adverse events accompanied the infusion of these cells in this trial after they had been transduced *ex vivo* by Enzo's vector HGTV43, the vector that will be used in this protocol.

We assayed for the presence of the anti-HIV-I genetic antisense RNA in bone marrow-derived stem cells (CD34+) as well as in CD4+ and PBMC over time in the trial subjects after infusion. The assay procedure included RT-PCR to amplify the antisense sequences from RNA followed by restriction enzyme digest of the amplified material to confirm the presence of the original antisense RNA.

To summarize, the results from the phase 1 clinical protocol demonstrate long term (6-12 months) survival of antisense RNA in a low number of bone marrow stem cells as well as cells in the peripheral blood mononuclear cell (PBMC) fraction and the CD4+ fraction. Since this low number of transduced PBMC has remained approximately constant over a number of months these data support the conclusion that stable engraftment of some of the antisense RNA-producing PBSC has occurred. The same conclusion can be drawn from the observation that bone marrow samples from 4 of the 5 subjects (the fifth did not yield enough bone marrow CD34+ cells to

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perform the assay.) Finally there was no evidence that multiple infusions (in 2 of the 5 patients) led to increased levels of engraftment.

In this amended protocol as a continuation of the trial reported above we propose to increase the amount of anti-HIV-1 genetic antisense RNA in CD4+ cells by increasing the number of CD4+ cells producing this RNA. We propose to isolate from circulation, a population of PBSC from HIV-1 infected subjects previously treated with G-CSF. After this isolation the subjects will initiate a treatment of immune conditioning using mycophenolate mofetil (MMF). The PBSC will be transduced with Enzo's triple U1/HIV-1 antisense MMLV vector (HGTV43.) After the transduction is completed the subjects will then be irradiated in an outpatient procedure (600 cGy, TBI), and the transduced cells containing the anti-HIV-1 antisense genes will be infused into the HIV-1 subject from which they were originally derived. The end points of this study are: the safety of the procedure; the extent of engraftment and proliferation of this engineered cell population. Our study will enroll up to 6 patients.